Genome stability: **Keeping the centrosome cycle on track**

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The protein kinase Mps1 and p53 both function in centrosome duplication and the spindle cell-cycle checkpoint. Defects in these functions can be potent sources of genomic instability by allowing mitosis to proceed with aberrant mitotic spindles.

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Genomic instability is characteristic of cancer cells, and promotes the accumulation of genetic lesions that contribute to their neoplastic progression. There are at least two general mechanisms by which cells exhibiting genomic instability can depart from the diploid state. One involves DNA damage from high levels of translocations, gene amplification or other chromosomal anomalies. The other involves chromosome segregation errors, probably arising from defective mitotic spindles, leading to extensive aneuploidy or even tetraploidy. A compelling argument has been made that cells with defective checkpoint controls on the cell cycle are unable to respond to DNA damage or spindle defects, thereby allowing the cells to proceed through the cell cycle while retaining the damage, possibly in consequence entering an aberrant state [1,2].

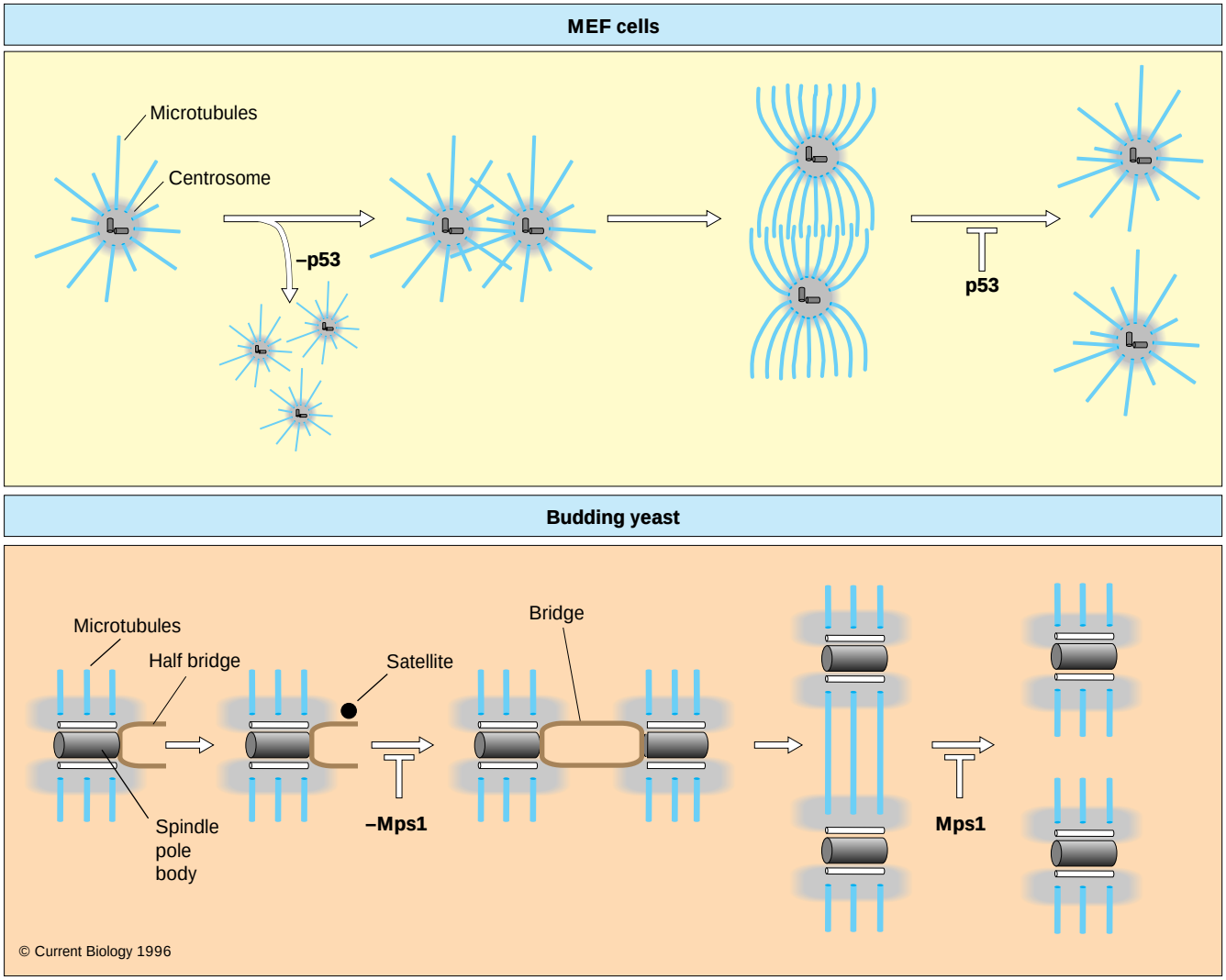
Checkpoint controls normally ensure that, as the cell cycle progresses, one process — DNA replication, for example — is successfully completed before a succeeding, incompatible one is begun. The tumor suppressor gene *p53* illustrates the importance of checkpoint controls in maintaining genomic integrity. The p53 protein functions in two cellular responses to DNA damage: the checkpoint that reversibly arrests the cell cycle at the G1/S transition to allow time for relatively mild DNA damage to be repaired before the genomic DNA is replicated; and the induction of cell death, or apoptosis, that occurs in the face of extensive DNA damage (reviewed in [1–3]). In fact, p53 has been so prominent in the analysis of the defective cell-cycle controls observed in cancer cells that it was named *Science*'s 1993 'molecule of the year'.

The initial sources of DNA damage or spindle malfunction that can lead to genetic lesions in the absence of checkpoint controls are not entirely known, but the damage could easily arise either from intrinsic errors in cellular processes or from environmental insult. Cellular damage and the failure to respond appropriately to the damage has been observed upon loss of function in an emerging class of cell-cycle regulators. These regulatory molecules have dual roles in cell-cycle progression and checkpoint function [4]. Generally, the two roles of these molecules are related, in that the molecule is involved in carrying out a process for which it also mediates the quality control *via* its checkpoint function. An excellent example is DNA polymerase ϵ in the budding yeast *Saccharomyces cerevisiae*, which is clearly required for DNA synthesis, but has a separable function in triggering the arrest of mitosis in response to incomplete DNA synthesis [5]. Clearly, defects in this class of cell-cycle regulators could be a potent source of genomic instability by both creating damage and then failing to respond to the damage.

Recent results have shown that the p53 can act as a dual function cell-cycle regulator. The surprising result, given p53's known activity in the G1 DNA-damage checkpoint, is that the newly defined roles of p53 are in mitotic spindle assembly [6] and in the spindle checkpoint that leads to a cell-cycle arrest in response to aberrant mitotic spindles [7]. The spindle checkpoint function was assayed by treating mouse embryonic fibroblast (MEF) cells with the microtubule poison, nocodazole. This drug causes defects in the spindle and blocks mitotic progression by triggering the spindle checkpoint, so that wild-type cells arrest with a post-S-phase DNA content. MEF cells derived from *p53*-null mutant mice, however, inappropriately reinitiate DNA synthesis in the presence of nocodazole, showing that the spindle checkpoint fails to function in these cells and implying that p53 is an essential component of the checkpoint machinery [7].

A separate study of MEF cells from *p53*-null mice has shown that they have a defect in centrosome duplication [6]. The cells appear to over-produce centrosomes, as shown by the appearance of several foci of γ tubulin staining, instead of the expected one or two, according to the cell-cycle stage. The additional foci may represent extra centrosomes, partially assembled centrosomes or centrosomal fragments. Nonetheless, the cells are clearly aberrant and their defective centrosome assembly has clearly generated multipolar spindles. The origin of these multipolar spindles was addressed using synchronized cells to show that the multiple γ tubulin foci could arise from a single defective round of centrosome duplication in G1 phase. This observation suggests that the multipolar spindles do not result from the mis-segregation of the centrosomes, but rather from a defect in centrosome duplication. Taken together, these two studies suggest that MEF cells lacking p53 often fail in centrosome assembly and go on to form aberrant spindles that may be allowed to mis-segregate

Figure 1



A schematic representation of centrosome duplication in a mouse cell (top) and a budding yeast cell (bottom), showing the defects in centrosome duplication that result from a loss of p53 or Mps1 activity and the second function both these proteins have in blocking cell-cycle progression if there is a defect in the mitotic spindle. (During the budding yeast cell cycle, the 'satellite' appears on the cytoplasmic face of the half bridge during spindle pole body duplication, and is thought to be the precursor of the new spindle pole body [9].)

chromosomes because the spindle assembly checkpoint is defective as well. Such a model implicates p53 in maintenance of the genome at the fundamental level of controlling centrosome duplication, an early stage of spindle assembly, and in the spindle checkpoint's assessment of the functionality of the spindle.

Both centrosome duplication and the spindle checkpoint have been studied in *S. cerevisiae*. The morphology of the yeast equivalent of the centrosome — the spindle pole body — and its duplication in G1 phase have been well described. Furthermore, several genes which are required for the proper execution of spindle-pole duplication have been identified by mutation (reviewed in [8,9]). Conditional loss of function in any one of these genes leads to failure in spindle pole body duplication, leaving the cell with a single functional spindle pole body and in some cases with a defective second spindle pole body. In general, these cells with single spindle pole bodies continue with DNA synthesis and bud growth, eventually coming to a mitotic arrest with a monopolar spindle.

The mitotic arrest observed in yeast cells with monopolar spindles is induced through the spindle checkpoint [10,11]. Several yeast genes involved in this checkpoint have been identified by mutations that cause cells to be unable to arrest in the presence of microtubule poisons, and the function of the products of these genes in a signaling

pathway is emerging [12]. These genes are required for cells to arrest in mitosis after failed spindle pole body duplication. The arrest is transient, however, as cells containing monopolar spindles have been observed to carry out cytokinesis and reinitiate DNA synthesis at the nonpermissive temperature, thus acquiring twice the ploidy of the starting strain [13].

This phenomenon of a transient mitotic arrest in cells containing a monopolar spindle may result from attenuation of the checkpoint pathway signal, or from fulfillment by the monopolar spindle of the criteria for release from the arrest state, which may be kinetochore attachment [12]. Regardless of the mechanism, these cells carry out a monopolar mitosis, demonstrating that cells defective in spindle pole body duplication can exhibit an increase in ploidy, one type of genomic instability. This situation is a variation on the initial hypothesis that genomic instability results from defective checkpoint control [1]. In this case, a functioning checkpoint eventually fails by allowing the cell cycle to proceed despite the presence in the cell of an aberrant structure.

Studies of the yeast monopolar spindle mutants have not only offered insights into cellular pathways to increased ploidy, but have also led to the identification of the *MPS1* gene, which acts both in spindle pole body duplication and the spindle checkpoint, and encodes a protein kinase [10,11,13]. This dual requirement for the Mps1 kinase is similar to that for p53 in centrosome duplication and the spindle checkpoint, though the roles of these two genes in spindle pole duplication are different (Fig. 1). Whereas the loss of p53 leads to over-replication or aberrant assembly of centrosomes [6], the loss of Mps1 activity leads to a block in spindle pole duplication [13].

Mps1 and p53 do, however, have comparable roles in the spindle checkpoint. Phenotypic analyses of cells lacking p53 [6] or active Mps1 [10] show that both proteins are required for the arrest elicited by nocodazole treatment. Although there are some phenotypic differences between cells lacking p53 or active Mps1, it appears that a regulatory link between centrosome duplication and the spindle assembly checkpoint may be general feature in eukaryotes. Defects in the genes that link these two pathways can be potent sources of genomic instability, because their failure to function not only leads to defects in spindle assembly, but also leaves the cell unable to respond appropriately to the defective spindle.

The p53 protein is nonfunctional in cells that give rise to many different types of cancer, but the contributions of defects in centrosome duplication and the spindle checkpoint to genomic instability in these cells remain to be determined. Despite the potential significance of these pathways in the maintenance of ploidy, they are largely uncharacterized in mammalian cells. The molecules that work with p53 in its centrosome assembly and spindle checkpoint functions need to be identified. Furthermore, the p53-independent pathways that allow some cell lines lacking p53 to arrest in the presence of nocodazole remain to be elucidated [7]. Continued analysis in yeast of spindle pole duplication, the spindle checkpoint pathway and the intersection of these pathways will augment our understanding of similar pathways in mammalian cells.

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